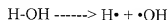


radical pair, the spins retain their original orientation $R\uparrow + \downarrow R'$ until the electron spins randomize over time. During the time the spins are paired, the radicals can recombine and revert to the starting material. If either of these paired radical spins should intersystem cross (ISC) to the triplet spin state ($R\uparrow + \uparrow R'$) they can no longer recombine until their spins are once again paired. That situation is preferred to release the bioactive agent from the bioconjugate. However, in healthy tissue, it is desired to prevent, or at least minimize, the cleavage of the conjugate. If that event, it is desirable to alter the rate of ISC by introducing an external magnetic field that increases the gap between the triplet state energy levels, thus encouraging the recombination of the original bioconjugate in the healthy tissues.

The recombination rate can be increased by application of a magnetic field in the range of 100-3000 gauss to the healthy tissues leading to a net decrease in the photochemical quantum yield and a decrease in drug release into healthy tissues by a factor of at least 2. Application of about 300 to 1000 gauss is considered to be optimal. See, Grissom (1995).

The bioconjugates of the present invention can undergo cleavage by ultrasound or sonolysis as follows. Although any non-reactive atom can be bound to the cobalt atom in the bioconjugate and cleaved by sonolysis, it is preferred that the atom be a carbon atom. The vitamin B₁₂ cofactor occurs naturally in two forms: adenosylcob(III)alamin, (AdoCbl^{III}), also known as coenzyme B₁₂ and methylcob(III)alamin, (CH₃Cbl^{III}). The remarkably weak C-Co bond from the corrin ring to the 5'-deoxyadenosyl or methyl ligand imparts a most unusual chemistry to cobalamins. The C-Co bond energy in AdoCbl^{III} and CH₃Cbl^{III} is estimated to be as low as 31 and 37 kcal/mole, respectively. This makes the C-Co bond one of the weakest covalent bonds known and allows sonolysis of the bond by the application of ultrasound in the range of about 20 kHz - 500 MHz, preferably 20 kHz - 100 MHz, more preferably 20 kHz to 10 MHz.

Sonolysis of aqueous solutions produces a high concentration of hydroxyl radicals and hydrogen atoms according to the equation:



These reactive oxidizing and reducing species are responsible for initiating most reactions in aqueous solvents. Ultrasound irradiation and sonochemistry are often not described as high-energy processes, but during sonolysis, the development, growth and implosion of bubbles in a liquid create extreme reaction environments on a microscopic scale. The collapse of cavitation

bubbles produces pressures >500 atm. and temperatures >5000°C. The radicals formed survive the collapse of the bubbles. The formation of hydroxyl radicals *in vivo* has been the focus of several investigations because of the potentially deleterious effects of oxidizing free radicals in human tissue. However, such radicals do not present an unacceptable health risk, as clinical
5 experience has demonstrated that diagnostic ultrasound is a benign procedure. The ability to form these radicals by sonolysis *in vivo* provides a mechanism for the triggered release of active neoplastic and other agents from conjugation with B₁₂, Co[SALEN] or other suitable cobalamin or cobalamin like substrates.

In the sonolysis of drug-B₁₂ conjugates (utilized here only as an example and not
10 intended to limit the invention), the C-Co bond is cleaved in aqueous solution to produce the drug and a cob(II)alamin (Cbl^{II}) under anaerobic conditions or the drug and aquocob(III)alamin (H₂O-Cbl^{III}) under aerobic conditions. The cleavage is not a direct breaking of the C-Co bond. Rather, under anaerobic conditions the predominant pathway for C-Co bond cleavage by sonolysis is through reduction of drug-Cbl^{III} to the labile drug-Cbl^{II} by H•, followed by dissociation to the closed-shell drug and Cbl^{II}. The reaction of HO• with drug-Cbl^{II} leads to H₂O-Cbl^{III}, as well as degradation of the corrin ring. Under aerobic conditions, the pathway for the C-Co bond cleavage by sonolysis is through reduction of the drug-Cbl^{III} to produce drug and Cbl^{II}, but O₂ oxidizes Cbl^{II} to H₂O-Cbl^{III}. In either event, sonolysis from the focused application
15 of ultrasound, results in C-Co bond cleavage and the irreversible release of the drug from the cobalamin. Therefore, sonolysis-triggered release can occur under aerobic and hypoxic conditions alike.

The present invention is useful in the treatment of (including, but not limited to) cancer, hepatitis, psoriasis and other localized diseases, as well as for gene therapy and peptide therapy.
25 The bioconjugates according to the present invention can be administered by any route, including intravenously, parenterally, orally, intramuscularly, intrathecally or as an aerosol. The mode of delivery will largely depend on the biological effect desired to be achieved. A skilled artisan will readily know the best route of administration for a particular treatment in accordance with the present invention. The appropriate dosages will depend on the route of administration
30 and the treatment indicated, and can be readily determined by a skilled artisan, such as by extrapolation from current treatment protocols. If the organocobalt complex of the bioconjugate

is cobalamin or derivative or analogue, it is preferred to administer orally a bolus of vitamin B₁₂ prior to administration of the bioconjugate to reduce or eliminate potential hepatotoxicity which might otherwise result from the administration of the bioconjugate. The oral dose of B₁₂ will saturate the enterohepatic shuttle system and load hepatocytes with cobalamin. It is preferred that 0.1 mg to 100 mg, more preferably 1.0 mg to 10 mg, of vitamin B₁₂ be administered prior to the administration of the bioconjugate containing cobalamin. In addition, vitamin B₁₂ can be administered, preferably intravenously, following the selective cleavage of bioconjugate to wash out all bioconjugate which has not been cleaved, and thus further reduce potential systemic effects. It is preferred that 0.1 mg to 100 mg, more preferably 10 mg to 100 mg, of vitamin B₁₂ in saline be administered intravenously over 4-5 hrs. Finally, prior to the administration of a cobalamin-based bioconjugate, nitrous oxide can be administered to the subject in order to deplete body stores of cobalamin in its various forms, such as methylcobalamin. Administration of nitrous oxide has the effect of creating a greater body deficit of cobalamin before administration of the cobalamin-based bioconjugate.

Pharmaceutical compositions containing a compound of the present invention as the active ingredient can be prepared according to conventional pharmaceutical compounding techniques. See, for example, *Remington's Pharmaceutical Sciences*, 17th Ed. (1985, Mack Publishing Co., Easton, PA). Typically, an antagonistic amount of active ingredient will be admixed with a pharmaceutically acceptable carrier. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral, parenteral, intrathecal, transdermal, or by aerosol.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by